

# Association of Inflammatory Cytokines with Traditional and Nontraditional Cardiovascular Risk Factors in Indians with known Coronary Artery Disease

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## Abstract

**Background:** Inflammatory processes are implicated in the etiology of cardiovascular disease (CVD). Data on the association of inflammatory markers with cardiovascular risk factors in Indian patients with CVD are limited. **Aim:** This study was conducted with the aim to evaluate the association of inflammatory markers with traditional and nontraditional cardiovascular risk factors in angiographically proven coronary artery disease (CAD) patients. **Subjects and Methods:** We studied the association of serum highly sensitive C-reactive protein (hsCRP) (0.1-37.9 mg/l), interleukin-6 (IL-6) (2-253.2 pg/ml) and tumor necrosis factor-alpha (TNF- $\alpha$ ) (8-525.8 pg/ml) with cardiovascular risk factors in 300 (M: 216, F: 84; mean age: 60.9 (12.4) years) CAD patients. All patients were evaluated for anthropometry and cardiovascular risk factors, and blood samples were collected for biochemical and inflammatory markers. Statistical analysis was carried out using SPSS Version 20. **Results:** Mean hsCRP, IL-6 and TNF- $\alpha$  in study population were 11.7 (9.7) mg/l, 64.5 (75.2) pg/ml, and 25.3 (40.9) pg/ml respectively. A total of 73.6% (221/300) patients had hsCRP levels >3.0 mg/l. All inflammatory markers were significantly higher and showed a positive correlation with dyslipidemia, diabetes mellitus, and/or hypertension (HTN). TNF- $\alpha$  had a negative correlation with age and positive correlation with smoking. Only IL-6 and hsCRP had a positive correlation with insulin resistance and negative correlation with insulin secretion. Among lipid parameters, triglyceride had a positive correlation, and high density lipoprotein had a negative correlation with all inflammatory markers. There was a progressive increase in the percentage of subjects with diabetes, HTN, and dyslipidemia with increasing levels of inflammatory markers. **Conclusions:** Indian patients with CAD had significantly high levels of inflammatory markers, which were related to cardiovascular risk factors.

**Keywords:** C-reactive protein, Coronary artery disease, Inflammatory markers, Interleukin-6, Tumor necrosis factor- $\alpha$

## Introduction

Epidemiologists have been sounding an alarm on the rapidly rising burden of cardiovascular disease (CVD) for the past 15 years in world over including India.<sup>[1]</sup> It was projected that

by 2020, CVD will be the largest cause of disability and death in India.<sup>[2]</sup> The etiology of CVD is multifactorial and certainly there are many traditional and nontraditional risk factors that are associated with CVD.<sup>[3,4]</sup> High incidence of CVD in Indians cannot be explained by traditional risk factors. Hence, many researchers investigated and reported other novel risk factors to explain the increasing incidence and prevalence of CVD that is Vitamin B12, homocysteine, inflammatory markers, macro and micronutrients and presence of metabolic syndrome.<sup>[3-7]</sup>

Past decades have witnessed a spurt in research activity that has shown a key role of inflammation in coronary artery disease (CAD) and other manifestations of atherosclerosis.

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Immune cells dominate early atherosclerotic lesions and their effector molecules accelerate progression of the lesions, and can elicit acute coronary syndromes.<sup>[6-9]</sup> The inflammatory process associated with atherosclerosis lead to increased blood levels of inflammatory cytokines and other acute-phase reactants. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. Interleukin-6 (IL-6) is an IL that acts as both a pro-inflammatory and anti-inflammatory cytokine.<sup>[5]</sup> C-reactive protein (CRP) is a member of the class of acute-phase reactants as its levels rise dramatically during inflammatory processes. IL-6 and TNF- $\alpha$  are inflammatory cytokines and the main inducers of the secretion of CRP in the liver.<sup>[6]</sup> CRP is a marker of low grade inflammation, and few studies suggest that this protein has a role in the pathogenesis of atherosclerotic lesions in humans.<sup>[10-15]</sup> Levels of CRP and IL-6 are elevated in patients with unstable angina and myocardial infarction (MI), with high levels predict worse prognosis.<sup>[8]</sup> Highly sensitive CRP (hsCRP) and IL-6 are also associated with increased risk of all-cause mortality, and their measurement improves the ability to predict the risk of cardiovascular events.<sup>[8,16-19]</sup> Inflammation and related cytokines also interact with various metabolic factors which are related to the atherosclerotic process such as hyperglycemia, dyslipidemia, and insulin resistance (IR).

In patients with CAD, variable association of inflammatory markers with traditional and nontraditional risk factors has been described in the literature.<sup>[20-26]</sup> Few studies have analyzed inflammatory markers and its association with CVD risk factors in subjects with CAD from India.<sup>[10,27,28]</sup> Sharma *et al.*<sup>[27]</sup> studied hsCRP, dyslipidemia and oxidative stress in a small group of premature CAD. Goswami *et al.*<sup>[28]</sup> evaluated interplay between dyslipidemia, hsCRP and TNF- $\alpha$  in patients with acute MI. These all studies have studied only one or two inflammatory markers and none have studied all markers simultaneously and their relationship with all traditional and nontraditional risk factors. Combination of inflammatory markers will help to know the importance of inflammatory markers in patients with CVD. Therefore, we conducted this study to evaluate the association of inflammatory markers namely serum hsCRP, IL-6 and TNF- $\alpha$  with traditional and nontraditional cardiovascular risk factors in angiographically proven CAD patients.

## Subjects and Methods

Three hundred patients with known CAD were included in this cross-sectional study. Patients, who were admitted in Cardiology Department of a single tertiary care center for evaluation of chest pain, and found angiography positive, were selected in the study consecutively. Exclusion criteria were the presence of chronic kidney disease; hepatic dysfunction; known endocrinal (except diabetes mellitus [DM]) or rheumatologic diseases or chronic infections. All cases were interviewed using a questionnaire, which included data on smoking, physical activity. Height, weight, waist, and hip

circumference were measured. Body mass index (BMI) was calculated by dividing weight in kg with square of height in meters. Waist hip ratio (WHR) was calculated. Data on clinical history of hypertension (HTN) and DM and medications was also acquired.

## Definitions

Traditional (conventional) risk factors were defined as follows: BMI < 25-normal,  $\geq 25$  overweight/obese, DM (by history and American Diabetes Association; 2011), HTN (systolic and diastolic blood pressures above 140 and 90 mm Hg, respectively). Dyslipidemia was defined as triglyceride level  $\geq 150$  mg/dl and HDL Cholesterol level <40 mg/dl (National Cholesterol Education Program Adult Treatment Panel III).

Initially, we thought of including the control group with normal coronary artery. However, we found that most of the patients had lesion ranging from 20% to <70% labeled as nonsignificant lesion. Hence, these patients if included as control, due to underlying pathology we will not be comparing our patients with individuals with absence of CAD. Moreover, age matched asymptomatic controls may not necessarily exclude underlying CAD. Hence, we have taken 25 asymptomatic apparently healthy young physically active controls from our previous study,<sup>[11]</sup> where only data for hsCRP was available.

Fasting blood samples were collected after 14 h fasting. Total cholesterol, triglyceride, and high density lipoprotein (HDL) were measured by using cholesterol oxidase para-aminoantipyrine, lipase glycerol kinase, the enzymatic reaction respectively, and low density lipoprotein (LDL), and very LDL (VLDL) were calculated by Freidwald formula. Inter assay 3.8%, and intra precision was 2% respectively for all biochemical parameters. TNF- $\alpha$  (linearity: 200 pg/ml, sensitivity: 2 pg/ml), IL-6 (linearity: 800 pg/ml, sensitivity 8 pg/ml), and hsCRP (linearity: 119.3 mg/l, sensitivity: 0.1 mg/l) were measured by enzyme linked immunosorbent assay method with kits manufactured by Gen-probe Diaclone, France and Biochek, CA, USA. Insulin was done by microparticle enzyme immunoassay with commercial kits supplied by Abbott laboratory, USA. Intra assay and inter assay precision was <5% and <10% respectively for above parameters. IR and sensitivity was calculated by using homeostatic model analysis (HOMA) model ( $\text{HOMA-IR} = \text{Fasting insulin } (\mu\text{IU/ml}) \times \text{Fasting glucose (mmol/l)} / 22.5$ ; and quantitative insulin check index (QUICKI) ( $\text{QUICKI} = 1 / (\log [\text{fasting insulin } \mu\text{IU/ml}] + \log [\text{fasting glucose mg/dl}])$  respectively. The study was approved by Institutional ethics committee. The study period was from 2009 to 2011. Informed consent was obtained from all subjects.

Sample size calculated with 95% confidence ( $1-\alpha$ ), 90% power ( $1-\beta$ ), population prevalence of 10% and relative risk of 2 was 268 subjects. Statistical analysis was carried out using

SPSS Version 20.0 (IBM, SPSS Inc. Chicago, USA). Data were presented as mean (standard deviation, median (range) or number (%) unless specified. Significance levels were analyzed by Student's *t*-test between two parameters and ANOVA test when parameters were more than two. If Bartlett's Chi-square test for equality of population variances was  $<0.05$ , then Kruskal–Wallis test was applied. Pearson correlation was used to evaluate the correlation between traditional and nontraditional risk factors such as markers of IR (Insulin, HOMA-IR, QUICKI) and inflammatory markers (hsCRP, IL-6 and TNF- $\alpha$ ). Multiple regression analysis was performed after adjustment for age, sex, BMI, and presence of HTN. All nonparametric data were analyzed by Chi-square test.  $P < 0.05$  was considered as statistically significant.

## Results

Three hundred patients with known CAD (male: 216; female: 84, mean age 60.9 [12.4] years, 95% confidence interval [CI] 59.50–62.34 years) were studied. Table 1 shows baseline characteristics of the subjects studied. Mean hsCRP, IL-6 and TNF- $\alpha$  in study population were 11.7 (9.7) mg/l (median 12 mg/l), 64.6 (75.3) pg/ml (median 25.0 pg/ml), and 25.3 (40.9) pg/ml (median 10.0 pg/ml), respectively. Mean hsCRP levels (age adjusted) were higher in study population than controls (2.0 (0.9) mg/L,  $P < 0.001$ ).

A total of 73.6% (221/300) of the patients had hsCRP levels  $>3.0$  mg/l. There was no gender difference in hsCRP levels [Table 2]. hsCRP, IL-6, and TNF- $\alpha$  levels were higher in the subject with dyslipidemia, diabetes, and/or HTN compared to nondiabetics and normotensives. It was positively correlated with total and LDL cholesterol, triglyceride, insulin levels, HOMA-IR, and negatively correlated with insulin secretion

and HDL cholesterol in univariate analysis [Table 3 and Figure 1]. However, after adjustment with other risk factors, the association between hsCRP and HTN was lost, but the significance was maintained with dyslipidemia and DM (HTN; beta coefficient 1.4,  $P = 0.15$ , DM; beta coefficient 2.5,  $P = 0.01$ , Dyslipidemia; beta coefficient 9.0,  $P < 0.001$ ). A similar observation was noted for total cholesterol, triglyceride, LDL, and VLDL with quartiles of hsCRP except HDL that decreased [Table 4].

Odds ratio of DM (1.70, 95% CI: 1.19–2.43), HTN (1.76, 95% CI: 1.28–2.4), and dyslipidemia (5.8, 95% CI: 3.0–11.3) increased from lowest to the highest quartile of hsCRP. A similar trends were seen with IL-6 (Odds ratio of DM [1.73, 95% CI: 1.18–2.52], HTN (1.65, 95% CI: 1.2–2.27), and dyslipidemia (4.5, 95% CI: 2.5–8.1)].

Subjects were also categorized into subject with modifiable risk factor (DM, HT, and dyslipidemia) and without modifiable risk factors. Subjects with modifiable risk factors had significantly higher inflammatory levels (hsCRP–12.8 [9.5] vs. 4.8 [7.2] mg/l, IL-6–72 [77.1] vs. 21.0 [42.5] pg/ml, TNF- $\alpha$  – 27.4 [43.5] vs. 12.5 [13.9] pg/ml) compared with without modifiable risk factors.

## Discussion

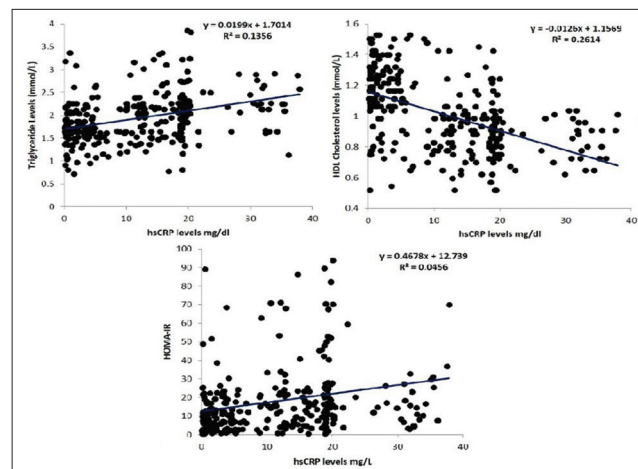
Atherosclerosis is a chronic inflammatory state and many studies have demonstrated an association of inflammatory markers with CAD. Elevated levels of these markers predict future ischemic events, and mortality from CAD.<sup>[5,12]</sup> In the present study, we have measured inflammatory markers in three hundred patients with angiographically proven CAD. Among inflammatory markers, hsCRP has been extensively studied and was part of two meta-analysis.<sup>[3,13]</sup>

Mean hsCRP levels were higher in present study than those reported in the literature<sup>[10,27]</sup> except a study from

**Table 1: Basic characteristics of study population**

Parameters	Mean (SD)		P
	Male (n=216)	Female (n=84)	
Age (years)	60.9 (12.3)	61.03 (12.9)	0.1
BMI (kg/m <sup>2</sup> )	27.6 (3.6)	28.5 (4.0)	0.07
Smoking, n (%)	82 (73.9)	29 (26.1)	0.67
WHR	0.92 (0.05)	0.91 (0.06)	0.11
DM, n (%)	86 (68.8)	39 (31.2)	0.36
HTN, n (%)	72 (75.8)	23 (24.2)	0.39
DM and HTN, n (%)	59 (63.4)	34 (36.6)	0.03
Dyslipidemia, n (%)	81 (65.3)	43 (34.7)	0.04
Cholesterol, mmol/L	4.5 (1.1)	4.6 (1.2)	0.55
Triglyceride, mmol/L	2.24 (0.51)	1.97 (0.54)	0.42
HDL, mmol/L	1.0 (0.23)	0.9 (0.23)	0.25
LDL, mmol/L	2.7 (1.2)	2.8 (1.3)	0.54
VLDL, mmol/L	0.86 (0.24)	0.88 (0.27)	0.63
Insulin, pmol/L	342.3 (288.2)	362.5 (334.7)	0.60
HOMA-IR	16.6 (17.01)	22.2 (28.6)	0.50
QUICKI	0.28 (0.039)	0.27 (0.047)	0.46

BMI: Body mass index, WHR: Waist hip ratio, DM: Diabetes mellitus, HTN: Hypertension, HDL: High density cholesterol, VLDL: Very low density cholesterol, LDL: Low density cholesterol, HOMA-IR: Homeostatic model analysis–insulin resistance, QUICKI: Quantitative insulin check index, SD: Standard deviation



**Figure 1:** Correlation of hsCRP levels with triglyceride, HDL and HOMA-IR

**Table 2: Inflammatory markers according to traditional risk factors**

Risk factors	hsCRP (mg/L)		IL-6 (pg/mL)		TNF- $\alpha$ (pg/ml)	
	Mean (SD)	P	Mean (SD)	P	Mean (SD)	P
Age						
≤65	11.4 (9.6)	0.57	61.7 (74.6)	0.41	27.3 (49.2)	0.95
>65	12.0 (9.8)		69.0 (76.3)		21.9 (21.3)	
Sex						
Male	11.8 (10.02)	0.57	62.3 (73.8)	0.42	23.8 (41.8)	0.32
Female	11.1 (8.72)		70.1 (78.9)		29.0 (38.1)	
Smoking						
Present	12.7 (9.6)	0.12	70.4 (74.7)	0.29	32.6 (56.3)	<0.01
Absent	11.0 (9.6)		61.0 (75.5)		20.9 (27.2)	
Physical inactivity						
Yes	11.4 (9.2)	0.72	65.4 (75.6)	0.86	25.1 (34.2)	0.99
No	11.8 (9.9)		63.9 (75.2)		25.3 (44.6)	
BMI						
≤25	10.8 (9.7)	0.42	54.6 (70.2)	0.23	24.2 (65.5)	0.17
>25	11.9 (9.6)		67.2 (76.4)		25.5 (30.9)	
WHR						
≤0.9	10.4 (9.0)	0.16	62.9 (75.9)	0.81	20.9 (21.8)	0.61
>0.9	12.1 (9.8)		65.2 (75.1)		27.1 (46.6)	
DM						
Present	14.4 (10.2)	<0.001	78.2 (78.4)	<0.01	25.7 (28.5)	0.01
Absent	9.6 (8.7)		54.7 (71.5)		24.9 (47.8)	
HTN						
Present	13.1 (9.3)	<0.001	73.1 (77.6)	<0.01	29.2 (48.6)	<0.001
Absent	9.1 (9.6)		50.0 (68.9)		18.6 (20.8)	
DM and HTN						
Present	14.9 (9.71)	<0.001	79.2 (77.5)	0.02	28.4 (31.0)	<0.001
Absent	10.1 (9.2)		57.9 (73.4)		23.8 (44.5)	
Dyslipidemia						
Present	17.3 (8.3)	<0.001	99.4 (76.5)	<0.001	34.8 (54.8)	<0.001
Absent	7.6 (8.4)		39.9 (63.9)		18.5 (25.0)	

BMI: Body mass index, WHR: Waist hip ratio, DM: Diabetes mellitus, HTN: Hypertension, hsCRP: Highly sensitive C-reactive protein, IL-6: Interleukin-6, TNF- $\alpha$ : Tumor necrosis factor-alpha, SD: Standard deviation

**Table 3: Correlation of inflammatory markers with risk factors**

Risk factors	hsCRP <i>r</i> value ( <i>P</i> value)	IL-6 <i>r</i> value ( <i>P</i> value)	TNF- $\alpha$ <i>r</i> value ( <i>P</i> value)
Age (years)	0.059 (0.31)	0.064 (0.26)	-0.147 (0.01)
BMI (kg/m <sup>2</sup> )	0.014 (0.80)	0.076 (0.19)	-0.028 (0.63)
WHR	0.037 (0.52)	-0.011 (0.84)	0.043 (0.46)
DM	0.244 (<0.001)	0.155 (<0.01)	0.010 (0.85)
HTN	0.201 (<0.001)	0.149 (0.01)	0.126 (0.02)
Dyslipidemia	0.492 (<0.001)	0.390 (<0.001)	0.196 (0.001)
Cholesterol	0.150 (<0.01)	0.188 (0.001)	-0.024 (0.68)
Triglyceride	0.368 (<0.001)	0.257 (<0.001)	0.135 (0.01)
HDL	-0.511 (<0.001)	-0.392 (<0.001)	-0.198 (0.001)
LDL	0.162 (<0.01)	0.195 (0.001)	-0.010 (0.85)
VLDL	0.368 (<0.001)	0.257 (<0.001)	0.135 (0.01)
Insulin	0.153 (<0.01)	0.105 (0.07)	0.044 (0.44)
HOMA-IR	0.214 (<0.001)	0.163 (<0.01)	0.035 (0.54)
QUICKI	-0.298 (<0.001)	-0.222 (<0.001)	-0.090 (0.11)

BMI: Body mass index, WHR: Waist hip ratio, DM: Diabetes mellitus, HTN: Hypertension, HDL: High density cholesterol, VLDL: Very low density cholesterol, LDL: Low density cholesterol, HOMA-IR: Homeostatic model analysis-insulin resistance, QUICKI: Quantitative insulin check index, hsCRP: Highly sensitive C-reactive protein, IL-6: Interleukin-6, TNF- $\alpha$ : Tumor necrosis factor-alpha

Brazil (male 18.2 [2.3] mg/l and female 12.0 [2.1] mg/l) in angiographically proven patients with CAD.<sup>[14]</sup> In the present study, 73.6% of the patients had hsCRP levels >3.0 mg/l, whereas other studies reported above this level in 50% patients of CAD.<sup>[3,15]</sup> This can be explained by differences in age, sex ratio, BMI, associated morbidities, and dietary differences among various populations studied. Dietary differences as high carbohydrate-fat and low protein, mineral, and fiber diet,<sup>[29]</sup> Vitamin B12 deficiency, and hyperhomocysteinemia<sup>[4]</sup> and hypomagnesaemia<sup>[30]</sup> are associated with inflammation in Indian patients. IR and associated metabolic syndrome is also associated with increased levels of inflammatory markers<sup>[15]</sup> and Indians have higher IR than other population.<sup>[31]</sup>

Interleukin-6 is the main inducer of hsCRP production and secretion from liver. It has been reported to predict future risk of CAD and mortality in patients with CAD.<sup>[16]</sup> Mean IL-6 level in the present study was similar to the level reported in a study from Pakistan in patients with CAD with diabetes and without diabetes.<sup>[17]</sup> Other studies have also reported higher level of IL-6 in patients with CAD.<sup>[8,16]</sup> Serum IL-6 levels are



**Table 4: Cardiovascular risk factors according to quartiles of hsCRP**

hsCRP (mg/L)	≤2.4	>2.4-12	>12-18.9	>18.9	P
Smoking (%)	24 (31.2)	27 (36)	27 (37.5)	33 (43.4)	0.47
OR (95% CI)		1.11 (0.78-1.57)	1.14 (0.81-1.62)	1.31 (0.92-1.86)	
P value		0.64	0.52	0.16	
Physical inactivity (%)	29 (37.7)	34 (45.3)	23 (31.9)	30 (39.5)	0.41
OR (95% CI)		1.17 (0.84-1.62)	0.88 (0.64-1.21)	1.03 (0.74-1.43)	
P value		0.42	0.57	0.94	
Overweight/obesity (%)	60 (77.9)	53 (70.7)	55 (76.4)	63 (82.9)	0.35
OR (95% CI)		0.82 (0.55-1.22)	0.95 (0.65-1.39)	1.16 (0.80-1.66)	
P value		0.4	0.97	0.56	
DM (%)	25 (32.5)	22 (29.3)	34 (47.2)	44 (57.9)	<0.01
OR (95% CI)		0.93 (0.66-1.29)	1.36 (0.96-1.92)	1.70 (1.19-2.43)	
P value*		0.8	0.09	<0.01	
HTN (%)	35 (45.5)	47 (62.7)	50 (69.4)	56 (73.7)	<0.01
OR (95% CI)		1.4 (1.02-1.92)	1.59 (1.16-2.17)	1.76 (1.28-2.4)	
P value		0.04	<0.01	<0.01	
DM and HTN	16 (20.8)	16 (21.3)	27 (37.5)	34 (44.7)	<0.01
OR (95% CI)		1.01 (0.68-1.49)	1.54 (1.01-2.35)	1.85 (1.19-2.85)	
P value		0.9	0.03	<0.01	
Dyslipidemia (%)	8 (10.4)	16 (21.3)	46 (63.9)	54 (71.1)	<0.001
OR (95% CI)		1.61 (0.89-2.91)	4.90 (2.55-9.4)	5.8 (3.0-11.3)	
P value		0.1	<0.001	<0.001	
Cholesterol (mmol/L)	4.37 (0.98)	4.64 (1.18)	4.73 (1.24)	4.81 (1.26)	0.10
		0.19	0.06	0.01	
Triglyceride (mmol/L)	1.73 (0.46)	1.75 (0.42)	2.04 (0.49)	2.2 (0.54)	<0.001
		0.74	<0.001	<0.001	
HDL (mmol/L)	1.16 (0.21)	1.08 (0.24)	0.91 (0.18)	0.86 (0.16)	<0.001
		0.01	<0.001	<0.001	
LDL (mmol/L)	2.4 (1.06)	2.71 (1.2)	2.88 (1.36)	2.93 (1.43)	0.05
		0.1	0.03	0.01	
VLDL (mmol/L)	0.79 (0.20)	0.80 (0.19)	0.93 (0.22)	1.01 (0.24)	<0.001
		0.74	<0.001	<0.001	
WHR	0.92 (0.06)	0.92 (0.06)	0.92 (0.06)	0.92 (0.05)	0.78
		0.46	0.92	0.53	
Insulin (pmol/L)	256.2 (231.9)	320.1 (250)	428.5 (407.6)	392.3 (267.3)	<0.01
		0.1	<0.01	0.001	
HOMA-IR	11.1 (13.4)	14.5 (14.2)	23.8 (27.8)	23.5 (23.3)	<0.001
		0.12	<0.001	<0.001	
QUICKI	0.3 (0.04)	0.28 (0.04)	0.27 (0.03)	0.26 (0.03)	<0.001
		0.02	<0.001	<0.001	

\*P value between lowest quartile and the column of quartile, mean (SD). BMI: Body mass index, WHR: Waist hip ratio, DM: Diabetes mellitus, HTN: Hypertension, HDL: High density cholesterol, VLDL: Very low density cholesterol, LDL: Low density cholesterol, HOMA-IR: Homeostatic model analysis-insulin resistance, QUICKI: Quantitative insulin check index; hsCRP: Highly sensitive C-reactive protein, CI: Confidence interval, SD: Standard deviation, OR: Odds ratio

increased in diabetic, and hypertensive subjects<sup>[24]</sup> compared with those without these morbidities. Bautista<sup>[18]</sup> reported that after adjusting for other risk factors; age, sex, and BMI, IL-6 was not significantly associated with HTN that is similar to the present study. Serum IL-6 levels were not correlated with age, BMI, WHR, and smoking in our study. There are contradictory reports of relation of IL-6 with age<sup>[9]</sup> BMI<sup>[9,15,19,20]</sup> and smoking.<sup>[8,9]</sup>

Tumor necrosis factor-alpha is a pro-inflammatory cytokine, which is prerequisite for initiation of inflammation and production of other cytokine. TNF- $\alpha$  has been associated with recurrent MI and cardiovascular death.<sup>[32]</sup> TNF- $\alpha$  was negatively correlated with age and was positively correlated

with smoking. Similar observation has been made by others.<sup>[33-34]</sup> Elevated plasma TNF- $\alpha$  level have been observed in obese compared to lean subjects,<sup>[9,23]</sup> which is in contrast to our study. In the present study, TNF- $\alpha$  level was significantly higher in subjects with dyslipidemia compared with those without it. TNF- $\alpha$  level showed a positive association with increased triglyceride and reduced HDL, but was not related to total and LDL cholesterol. Mendall *et al.*<sup>[20]</sup> also observed a similar association. In contrast, Khan *et al.*<sup>[24]</sup> reported a strong association of TNF- $\alpha$  with LDL, but not with triglyceride and HDL cholesterol, whereas Goswami *et al.*<sup>[28]</sup> did not find any correlation with any lipid parameter. The reason for a strong association of TNF- $\alpha$  with a reduced serum

level of HDL is uncertain. It has been speculated that the reduced HDL seen in the inflammation result from increased serum concentration of serum amyloid A protein replacing apo-A1 as an apolipoprotein in HDL particles and that this leads to increased catabolism.<sup>[35]</sup> In experimental studies administration of TNF- $\alpha$  administration into mice and human increased triglyceride concentration by ~85%.<sup>[36]</sup> TNF- $\alpha$  increases plasma triglyceride by increasing the concentration of free fatty acids, which acts as substrate for triglyceride synthesis, and by diminishing the clearance of triglyceride rich lipoproteins (VLDLs) from the circulation.<sup>[36]</sup>

In this study, patients without modifiable risk factors (smoking, DM, HT, and dyslipidemia), had higher hsCRP levels than reported in healthy young Indian population.<sup>[27-28]</sup> This may suggest role of inflammation in patients without modifiable risk factors.<sup>[5,15]</sup> Low grade inflammation in these patients may be related to subclinical infection,<sup>[9]</sup> dietary factors<sup>[29]</sup> or environmental factors.<sup>[37]</sup> Genetic and epigenetic factors have also been implicated in inflammation. DNA methylation is emerging as a primary regulator of inflammation. DNA methylation states may vary over an individual's lifetime, and have been shown to regulate biological processes underlying CVD, such as atherosclerosis, inflammation, HTN, and diabetes.<sup>[38]</sup> Altered DNA methylation may be related to dietary factors such as sufficiency of folic acid in presence of vitamin B12 deficiency in Indian population.<sup>[39]</sup>

The main strength of the study is that all subjects had angiographically proven CAD and all inflammatory markers were simultaneously measured along with traditional and nontraditional risk factors. There were some limitations of our study. First, we have not taken a control group. We wanted to study population with confirmed CAD, and healthy asymptomatic controls do not certainly means absence of underlying asymptomatic CAD. Second, being a cross-sectional study, long-term follow-up data were not available. Third, males were more than females as CAD is more common in males, this may have affected results. Finally, it was single center study.

## Conclusion

the present study showed that inflammatory markers (hs-CRP, IL-6 and TNF- $\alpha$ ) have a strong association with diabetes, dyslipidemia, HTN, and IR in subjects with underlying CVD. The current study suggests that Indians have an underlying proinflammatory state, which may contribute to increased cardiovascular risk factors and predispose them to CVD. These markers may identify the future risk of CAD in Indian subjects if strong association is confirmed by future longitudinal prospective studies.

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